

# TRANSMISSION OF LA CROSSE VIRUS BY FOUR STRAINS OF *Aedes albopictus* TO AND FROM THE EASTERN CHIPMUNK (*TAMIAS STRIATUS*)

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**ABSTRACT.** Eastern chipmunks were successfully infected with La Crosse virus by bites of 3 New World strains of *Aedes albopictus* infected orally or transovarially. The virus was subsequently passed from the chipmunks to *Ae. albopictus*, POTOSI strain, and *Ae. triseriatus*. The chipmunks developed viremias of 1–4 days duration and antibody titers were similar in intensity and duration to those reported in chipmunks infected by *Ae. triseriatus*. After feeding on viremic chipmunks, *Ae. albopictus* became infected and transmitted La Crosse virus at rates similar to the native vector, *Ae. triseriatus*. *Aedes albopictus* transmitted La Crosse virus transovarially to first gonotrophic cycle offspring.

## INTRODUCTION

The Asian Tiger Mosquito [*Aedes albopictus* (Skuse)] was established in the continental United States in used tire piles near Houston, TX, by 1985 (Sprenger and Wuithiranyagool 1986). This species has since spread throughout the southeastern and eastern USA as far north as Chicago, IL, and Dover, DE (Nawrocki and Hawley 1987, Wesson et al. 1990). *Aedes albopictus* is a serious diurnal biting pest mosquito, but more importantly, it is an important vector of dengue in southeast Asia and a demonstrated vector of other arboviruses including chikungunya virus, Japanese encephalitis virus and La Crosse (LAC) virus in the laboratory (references in Hawley 1988). In the United States, *Ae. albopictus* now occurs within the range of LAC virus and at one site in Indiana is in a confirmed LAC virus zoonotic area where 3 of 5 wild-collected, engorged *Ae. albopictus* had fed on sciurids, probably eastern chipmunks [*Tamias striatus*] (Cully et al. 1991).

In the laboratory, *Ae. albopictus* can acquire LAC virus through membrane feeding and can subsequently transmit LAC virus to suckling mice (Grimstad et al. 1989). *Aedes albopictus* can also transmit LAC virus transovarially (Tesh and Gubler 1975, Streit and Grimstad 1990). We report preliminary studies to determine the feasibility of a more quantitative analysis of the LAC virus transmission potential of *Ae. albopictus*. Transmission of LAC virus to eastern chipmunks by 4 strains of *Ae. albopictus*, including one transovarially infected strain (OAHU), is described. We also document first gonotrophic cycle transovarial transmission of LAC virus in *Ae. albopictus*. The proportions of *Ae. albopictus*

and *Aedes triseriatus* (Say) that transmitted virus after blood feeding on viremic chipmunks are also estimated.

## METHODS

Eastern chipmunks were collected from 3 sites in St. Joseph County, IN, in 1989: Potato Creek State Park (September 12), Mishawaka (September 30), and the University of Notre Dame (October 5). By autumn of their first year, eastern chipmunks reach adult weights so we did not distinguish between adults and juveniles. At the time of capture, blood samples were collected by cardiac puncture to test for pre-existing neutralizing antibodies to LAC, Jamestown Canyon (JC), and trivittatus (TVT) viruses. The chipmunks were housed individually at 22°C, 60% RH and a 14:10 LD photoperiod throughout the experiment. *Aedes albopictus* and *Ae. triseriatus* were taken from colonies at the University of Notre Dame and maintained throughout the experiment at 27°C, 80% RH and a 16:8 LD photoperiod. Three strains of *Ae. albopictus* were used to infect chipmunks (see below). A fourth strain of *Ae. albopictus*, POTOSI, and *Ae. triseriatus* were subsequently allowed to feed on the infected chipmunks to determine oral infection rates from eastern chipmunks to mosquitoes. A strain of LAC virus, GW-1978, originally isolated from *Ae. triseriatus* in Indiana (Pinger et al. 1983), was used in its third suckling mouse brain passage.

OAHU *Ae. albopictus* were originally collected by Rosen in Hawaii prior to 1971. OAHU have been maintained at Notre Dame as a laboratory colony since 1971. INDY *Ae. albopictus* were collected at Indianapolis, IN in 1986 by R. Sinsco. The TEREZA strain of *Ae. albopictus* was collected by F. Antunao at Santa Tereza, Espirito Santo, Brazil in 1987. The POTOSI strain was collected at Potosi, MO by M. Niebylski in July 1989. The INDY, TEREZA and POTOSI

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mosquitoes were all in their third or less laboratory generation. *Aedes triseriatus* WALTON strain were collected at South Bend, IN, in 1969 by R. Beach. This strain has been reared in the laboratory since, and although it has developed several characteristics that are not typical of wild *Ae. triseriatus*, the strain has been used extensively in arbovirus research, so we used it as a standard for comparison with other studies.

**Transmission of virus to chipmunks:** In a preliminary step, 3 strains of *Ae. albopictus* (OAHU, INDY and TEREZA) were orally infected by a membrane feeder and tested for virus transmission in trials with suckling mice as described by Grimstad et al. (1989). The putative transovarially infected progeny of the infected OAHU females were similarly tested for virus transmission. The mosquitoes that successfully transmitted virus to mice were pooled by strain and route of infection (oral or transovarial) and allowed to refeed on chipmunks anesthetized with 1 cc Nembutal. The number of infected mosquitoes that fed on the chipmunks varied, but in every case more than 3 were engorged. Blood samples were drawn from the chipmunks before and after exposure to infected mosquitoes to monitor the development of viremia and the antibody response. During the first 10 days, retro-orbital blood samples were collected in unheparinized capillary tubes (0.07 ml), diluted to 0.7 ml with phosphate-buffered saline (pH 7.2), triturated, and stored at  $-70^{\circ}\text{C}$  for later assay. Thereafter, 0.5 ml volumes were drawn by cardiac puncture at weekly intervals for 2 months and then at approximately monthly intervals for 14 months. The sera from these samples were stored at  $-70^{\circ}\text{C}$ . Two experimental control chipmunks (754 and 766) were exposed to uninfected mosquitoes; chipmunk 754 was later inoculated subcutaneously with  $3.5 \log_{10}$  plaque forming units (PFU) of LAC virus and monitored for viremia and antibody response.

In an attempt at reinfection, chipmunk 778 was subcutaneously inoculated with  $3.5 \log_{10}$  PFU of LAC virus 14 months after the primary infection. Blood samples were collected daily for 7 days, at 2 weeks, and at 4 weeks post-inoculation to monitor subsequent viremia and anamnestic antibody response.

Viral titers calculated as  $\log_{10}$ PFU/ml (logs) in whole chipmunk blood were determined using Vero cell monolayers in 6-well plates. Samples were assayed in triplicate. After 4 days of incubation at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ , the monolayers were fixed and stained in a formalin-crystal violet solution. The number of PFU/ml were determined for wells containing 5–60 plaques, and the geometric mean titer (GMT) of these values was derived. Neutralizing antibody titers

against LAC, JC and TVT viruses were determined in triplicate using a microtiter serum dilution method (Pantuwatana et al. 1972).

**Infection of mosquitoes by viremic chipmunks:** The chipmunks described above were used as the sources of infectious blood in this experiment. On days 2 through 4 following LAC virus infected mosquito exposure, the chipmunks were anesthetized and made available for feeding by uninfected *Ae. albopictus* POTOSI and *Ae. triseriatus* WALTON. A minimum of 5 mosquitoes of each species fed at each exposure. After the mosquitoes fed on viremic chipmunks they were held together in 4 liter rearing cages and allowed to lay their first gonotrophic cycle eggs under standard rearing conditions. Fourteen days after taking the infectious blood-meal, mosquitoes were individually tested for oral transmission to suckling mice. We did not test for midgut or disseminated infections. *Aedes albopictus* POTOSI and *Ae. triseriatus* that transmitted LAC virus were pooled separately and allowed to refeed on seronegative chipmunks (778 and 776, respectively). The infection of these last 2 chipmunks meant, in effect, that 2 successive cycles of mosquito-chipmunk transmission were monitored over the course of these experiments.

**Transovarial transmission:** We assayed pools of adult female, first gonotrophic cycle offspring of female *Ae. albopictus* POTOSI that fed on chipmunk 693 (8 pools, 33 females each) and 769 (6 pools of 33 females). Pools of mosquitoes were allowed to feed on litters of suckling mice. Virus was confirmed in dead suckling mice by Vero cell culture as described above.

## RESULTS

**Chipmunk viremia:** All 4 chipmunks fed upon by the initial 4 groups of LAC virus infected *Ae. albopictus* developed viremia. The lowest and shortest viremia (2.95 logs, post-infection day 3) appeared in chipmunk 696, infected by *Ae. albopictus* INDY. Orally infected *Ae. albopictus* OAHU caused a 2-day (days 2 and 3 post-exposure in chipmunk 693) viremia (maximum titer 4.08 logs), while transovarially infected members of this strain produced a 4-day viremia (days 2–5 in chipmunk 769) and the highest virus titer recorded (4.87 logs on day 3). The 2 chipmunks infected by *Ae. albopictus* POTOSI (chipmunk 776, 3 days viremia, maximum 3.94 logs) and *Ae. triseriatus* (chipmunk 778, 3 days viremia, maximum 4.69 logs) had viremias within the range of the initial 4 chipmunks. Viral titers in the subcutaneously inoculated control chipmunk were similar (days 2–4, maximum titer 4.71 logs). No viremia was detected in chipmunk 778 following reexposure, or in the unexposed control chipmunks.

**Chipmunk antibodies:** All 6 infected chipmunks developed neutralizing antibodies to LAC virus within 5 days. Antibodies were observed only after the disappearance of detectable circulating virus in 4 chipmunks (693, 696, 776 and 778), while measurable levels of antibody and virus coexisted briefly in 769 and 789. Antibody titers generally increased through day 10 and peaked by day 31. The highest titer recorded was 1:640. The anamnestic response in chipmunk 778 was rapid, rising from 1:80 to 1:320 in 2 weeks, and appeared to have prevented viremia. The control chipmunks, exposed to uninfected *Ae. albopictus*, remained seronegative.

**Infection of mosquitoes:** All 4 chipmunks infected blood-seeking mosquitoes. Because we did not determine the numbers of non-transmitting mosquitoes with midgut or disseminated infections, the following estimates of transmission to mosquitoes may be low. Three of the 4 viremic chipmunks infected *Ae. albopictus* POTOSI and 3 of 4 infected *Ae. triseriatus* WALTON (Table 1). Of the 12 trials where chipmunk viral titers exceeded 2 logs, infections with subsequent transmission occurred in seven. In those 7 trials, 10 of 28 (36%) *Ae. albopictus* and 5 of 28 (18%) *Ae. triseriatus* demonstrated virus transmission to suckling mice. The difference in oral transmission rates between the mosquito species was not significant (Chi-square with Yate's correction = 1.46,  $P = 0.23$ ). There was no transmission from mosquitoes when they had fed on chipmunks with viral titers below detectable limits or when virus and antibody occurred simultaneously.

**Transovarial transmission:** We assayed adult offspring from first gonotrophic cycle eggs of

pools of female *Ae. albopictus* fed on chipmunks 693 and 769. Two of 8 pools of 33 female offspring from females fed on chipmunk 693 and 2 of 6 pools (33 females each) from females fed on chipmunk 769 were LAC virus positive at gonotrophic cycle 1. Because these eggs were laid by pools of females, we could not determine TOT or filial transmission rates from these data.

## DISCUSSION

*Aedes albopictus* is capable of orally transmitting LAC virus to eastern chipmunks, a species they feed on naturally in the wild (Cully et al. 1991). All 4 strains of *Ae. albopictus* caused chipmunk viremias similar to those reported in laboratory studies with *Ae. triseriatus* (Patrican et al. 1985). Because the study design was unreplicated by mosquito strain and the infective doses were not controlled, we cannot attribute differences between chipmunk infections to differences among mosquito strains.

For the native LAC vector, *Ae. triseriatus*, Patrican et al. (1985) found that most chipmunks infected by mosquito bite had infections lasting 3 days. Similarly, Pantuwatana et al. (1972) observed that most chipmunk infections were measurable over 2 days. While our virus assay was different (cell culture rather than suckling mouse brains), our results suggest that the duration of viremia effective for transmission may be longer than previously reported, 3–4 days rather than 2–3 days.

The primary antibody response to LAC virus infection in chipmunks remained relatively high during the 16-month study. The single attempt at reinfection failed to cause a measurable cir-

Table 1. Oral transmission of La Crosse virus from *Aedes albopictus* POTOSI strain and *Ae. triseriatus* to suckling mice after feeding on viremic chipmunks

Chipmunk no.	Infection source	Species of <i>Aedes</i>	Day post-infection	Chipmunk $\log_{10}$ PFU/cc	No. fed	No. transmitting to suckling mice
693	OAHU	<i>albopictus</i>	3	4.04	10	2
693	OAHU	<i>triseriatus</i>	2	4.08	3	1
693	OAHU	<i>triseriatus</i>	3	4.04	5	0
696	INDY	<i>albopictus</i>	3	2.95	10	2
769	OAHU	<i>albopictus</i>	2	2.00	1	0
769	OAHU	<i>triseriatus</i>	2	2.00	3	0
769	OAHU	<i>albopictus</i>	4	3.30	2	0
769	OAHU	<i>triseriatus</i>	4	3.30	15	1
789	TEREZA	<i>albopictus</i>	2	3.20	2	2
789	TEREZA	<i>albopictus</i>	3	4.43	6	4
789	TEREZA	<i>triseriatus</i>	3	4.43	5	3
789	TEREZA	<i>albopictus</i>	4	3.62	2	0*
789	TEREZA	<i>triseriatus</i>	4	3.62	10	0*
789	TEREZA	<i>albopictus</i>	5	3.30	9	0*

\* Virus in blood was accompanied by positive antibody titers.

culating viremia, but it did cause higher antibody titers, suggesting that the immune response effectively blocks LAC infection *in vivo*. Based on our serologies, primary and secondary LAC infections stimulate antibodies that neutralize JC and TVT viruses *in vitro*, although the responses against LAC virus are at least 4 times more effective. Similar heterologous responses were reported by Pantuwatana (1972) for artificially infected chipmunks.

All chipmunks had viremias sufficient to infect mosquitoes over a 1–4 day period. *Aedes albopictus* infected by viremic chipmunks transmitted LAC virus at a rate similar to that of the native vector *Ae. triseriatus*. Thirty-six percent of the *Ae. albopictus* that fed on chipmunks with viremias greater than 2 logs subsequently transmitted virus to suckling mice. This is comparable to 18% of *Ae. triseriatus*. Finally, transovarial transmission of LAC virus in the first gonotrophic cycle of *Ae. albopictus* is potentially important to this species' ability to maintain a LAC virus cycle in nature. We caution against overinterpretation of this last observation, however, because our samples are too small to allow adequate quantitative analysis.

Our observations are of interest because they demonstrate that *Ae. albopictus* is capable of oral transmission to and from the native LAC virus amplification host, the eastern chipmunk, and because they demonstrate that the species can transmit virus both orally and transovarially after acquiring infections from chipmunks.

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